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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/761,893	01/17/2001	Shih-Chieh Hung	11709-003001	6011

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EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	PAPER NUMBER
1636	
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08/31/2011	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)
	09/761,893	HUNG ET AL.
	Examiner	Art Unit
	Jennifer Dunston	1636

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 July 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4,6,9-20,34-38 and 42-45* is/are pending in the application.

4a) Of the above claim(s) *12-20 and 43-45* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 4, 6, 9-11, 34-38 and 42* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *17 January 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)	
1) ☐ Notice of References Cited (PTO-892)	4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)	5) ☐ Notice of Informal Patent Application
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.	6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/19/2011 has been entered.

Receipt is acknowledged of an amendment, filed 7/19/2011, in which claim 1 was amended, and claims 42-45 were newly added.

It is noted that the amendment to the claims filed on 7/19/2011 does not comply with the requirements of 37 CFR 1.121(c) because the claims were not submitted with markings to indicate the changes that have been made to the immediate prior version of the claims. The amendment filed 6/14/2011 was not entered. Thus, all changes should have been made relative to the amendment filed 5/3/2011, which was entered. In claim 1, the phrase "by recovering with trypsin-EDTA" in step (d) should have been underlined. Furthermore, claims 42-45 should have been provided with the status identifier "New." Amendments to the claims filed on or after July 30, 2003 must comply with 37 CFR 1.121(c). In the interest of compact prosecution, the claims filed 7/19/2011 have been entered.

Claims 1, 4, 6, 9-20, 34-38 and 42-45 are pending in the instant application.

Election/Restrictions

Applicant elected Group I without traverse in the reply filed on 9/4/2001.

New claims 43-45 read on the invention of Group II as set forth in the restriction requirement mailed 8/7/2001.

Claims 12-20 and 43-45 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 9/4/2001.

Currently, claims 1, 4, 6, 9-11, 34-38 and 42 are under consideration.

Claim Objections

Claim 42 is objected to because of the following informalities:

The wording of the phrase "wherein upper plate with pores of about 0.4 to 16 microns in diameter so that the small-sized cells can pass through the pores in the upper plate to reach the lower plate base before adhering" can be improved. Claim 42 depends from claim 1, which already requires the small-sized cells to adhere to the lower plate base following passing through the pores in the upper plate. Thus, the phrase "so that the small-sized cells can pass through the pores in the upper plate to reach the lower plate base before adhering" is unnecessary. It would be remedial to amend the claim to recite "wherein the pores are about 0.4 to 16 microns in diameter. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the fourth paragraph of 35 U.S.C. 112:

> Subject to the following paragraph, a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claims 36 and 37 are rejected under 35 U.S.C. 112, fourth paragraph, as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. This is a new rejection.

Claim 36 indirectly depends from claims 35 and 1. Claim 35 requires the mesenchymal stem cells to be cultured to confluence in step (b) of claim 1, when the mesenchymal stem cells adhere and are cultured. Claim 1 comprises "(d) collecting mesenchymal stem cells from the upper plate by recovering with trypsin-EDTA." Thus, claim 35 requires the step of collecting (i.e., recovering) the mesenchymal stem cells cultured until confluence by recovering with trypsin-EDTA. Accordingly, claim 36 does not further limit claim 35.

Claim 37 depends from claim 36 and requires the mesenchymal stem cells to be recovered by using trypsin-EDTA. However, recovering by using trypsin-EDTA is a limitation already present in claim 1. Thus, claim 37 does not further limit claims 1, 35 or 36.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 42 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new matter rejection**.

In the amendment filed 7/19/2011, new claim 42 was added. The claim requires the upper plate to have pores "of about 0.4 to 16 microns in diameter."

The originally filed specification discloses pore size ranges from about 0.4 to 40 microns in diameter (e.g., page 7, lines 27-29; page 8, lines 29-30). The specification does not specifically disclose the range of 0.4 to 16 microns in diameter. The specification does not exemplify the use of a pore size of 16 microns in diameter. Thus, the specification does not provide support for the subgenus range of about 0.4 to 16 microns in diameter.

The reply filed 7/19/2011 points to 37 CFR 1.57(a) and provides a certified copy of the foreign priority application, where it is asserted that a pore size of around 0.4 to 16 microns is disclosed. Thus, the reply asserts that no new matter is added.

In the present application, Applicant cannot rely on 37 CFR 1.57(a) to claim a pore size of about 0.4 to 16 microns. 37 CFR 1.57(a) has conditions that must be met before applicant can add omitted material to an application. See MPEP 201.17(II). The following conditions and requirements need to be met for an applicant to add omitted material to an application pursuant to 37 CFR 1.57(a):

(A) the application must have been filed on or after September 21, 2004;

(B) all or a portion of the specification or drawing(s) must have been inadvertently omitted from the application;

(C) a claim under 37 CFR 1.55 for priority of a prior-filed foreign application, or a claim under 37 CFR 1.78 for the benefit of a prior-filed provisional, nonprovisional, or international application, must have been present on the filing date of the application;

(D) the inadvertently omitted portion of the specification or drawing(s) must be completely contained in the prior-filed application;

(E) applicant must file an amendment to include the inadvertently omitted portion of the specification or drawing(s) within any time period set by the Office, but in no case later than the close of prosecution as defined by 37 CFR 1.114(b), or abandonment of the application, whichever occurs earlier;

(F) if the application is not otherwise entitled to a filing date, applicant must also file a petition under 37 CFR 1.57(a) accompanied by the petition fee set forth in 37 CFR 1.17(f);

(G) applicant must supply a copy of the prior-filed application, except where the prior-filed application is an application filed under 35 U.S.C. 111;

(H) applicant must supply an English language translation of any prior-filed application that is in a language other than English; and

(I) applicant must identify where the inadvertently omitted portion of the specification or drawing(s) can be found in the prior-filed application.

In the instant case, Applicant has not met the conditions and requirements of 37 CFR 1.57(a). The present application was not filed on or after September 21, 2004. The filing date of the present application is January 17, 2001. Furthermore, no English translation of the foreign priority document has been provided.

The original specification, drawings and claims were thoroughly reviewed and no support could be found for the amendment. Accordingly, the amendment is a departure from the specification and claims as originally filed, and the foreign priority document was not

incorporated by reference on the filing date of the present application and cannot provide support.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 4, 6, 9, 11, 34-38 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Prockop et al (US Patent No. 7,374,937 B1, effective date March 14, 2000, cited in a prior action; see the entire reference) and Matsui et al (US Patent No. 4,871,674, cited in a prior action; see the entire reference). This rejection has been rewritten to address the amendments to the claims in the reply filed 7/19/2011.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the

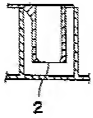
mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Prockop et al teach that RS cells can be separated from non-RS mesenchymal stem cells by ultrafiltration. Prockop et al teach that smaller RS cells will pass through an ultrafiltration membrane having appropriately sized pores, and such a membrane is a Millipore brand 10 micrometer isopore polycarbonate (plastic) membrane (e.g., column 39, line 60 to column 40, line 42).

Matsui et al teach culturing cells in a cell culture device comprising a cell culture insert comprising a membrane filter (2) on the bottom of the culture cell, which is composed of polycarbonate (e.g., column 2, lines 41-55; column 3, lines 5-18). The culture device is shown in Figure 8, which is reproduced below:

Fig. 8



It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include

the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach that mesenchymal stem cells adhere to plastic for culturing, and Caplan et al teach it is within the ordinary skill in the art to use a filter to remove fat cells and red blood cells from cells of bone marrow. Furthermore, Prockop et al teach the collection of mesenchymal stem cells on a filter of polycarbonate containing 10 micrometer pores, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Prockop et al (US Patent No. 7,374,937 B1, effective date March 14, 2000, cited in a prior action; see the entire reference) and Matsui et al (US Patent No. 4,871,674, cited in a prior action; see the entire reference) as applied to claims 1, 4, 6, 9, 11, 34-38 and 42 above, and further in view of Pittenger et al (Science, Vol. 284, pages 143-147, 1999, cited in a prior action; see the entire reference).

This rejection has been rewritten to address the amendments to the claims in the reply filed 7/19/2011.

The combined teachings of Caplan et al, Prockop et al, and Matsui et al are described above and applied as before.

Caplan et al, Prockop et al, and Matsui et al do not specifically teach that the mesenchymal stem cells are CD34-.

Pittenger et al teach the isolation of human mesenchymal cells from bone marrow taken from the iliac crest (e.g., page 143, right column). Pittenger et al teach that the mesenchymal stem cells are CD34- (e.g., paragraph bridging pages 143-144). The mesenchymal stem cells isolated by Pittenger et al are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to specifically use a bone marrow aspirate from human iliac crest, because Caplan et al and Pittenger et al teach the use of bone marrow from iliac crest to isolate mesenchymal stem cells that are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute iliac crest bone marrow for any other type of bone marrow to achieve the predictable result of recovering CD34- mesenchymal stem cells that are also capable of differentiating to adipose, cartilage or bone tissue.

Claims 1, 4, 6, 9, 11, 34-38 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire

reference) in view of Burkitt et al (Wheater's Functional Histology (1993), page 60, cited in a prior action) and Mussi et al (US Patent No. 5,409,829, cited in a prior action; see the entire reference). This rejection has been rewritten to address the amendments to the claims in the reply filed 7/19/2011.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach

plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Burkitt et al teach that red blood cells are 6.7-7.7 μm in diameter and nucleated cells have a diameter greater than 7.7 μm (page 60).

Mussi et al teach the introduction of a mixture of cells to be grown into a culture chamber in a suitable growth medium (e.g., column 2, lines 46-50). Mussi et al teach that the cells are grown in a culture insert contained within a well, where the insert is suspended in the well (e.g., paragraph bridging columns 3-4; Figure 4). The culture insert contains a membrane (20), which may be formed from a polymeric material such as polyethylene terephthalate, polycarbonate, and the like with open pores throughout (e.g., column 3, lines 50-53). Mussi et al teach that the pores are between about 0.2 to about 10 microns in diameter (e.g., column 3, lines 53-57).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells in

medium into the culture insert of the culture device of Mussi et al because Caplan et al teach it is within the ordinary skill in the art to use a filter to remove red blood cells from cells of bone marrow aspirate and Mussi et al teach the use of a porous polycarbonate filter membrane, where the pore diameter can be about 0.2 to about 10 microns in diameter, and Burkitt et al teach that red blood cells are the size which would pass through the filter of Mussi et al while nucleated mesenchymal stem cells of Caplan et al would be retained on top.

One would have been motivated to make such a modification in order to provide an enriched population of mesenchymal stem cells without the extra steps of using a column containing a filter, or other separation method, as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Mussi et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Burkitt et al (Wheater's Functional Histology (1993), page 60, cited in a prior action) and Mussi et al (US Patent No. 5,409,829, cited in a prior action; see the entire reference) as applied to claims 1, 4, 6, 9, 11, 34-38 and 42 above, and further in view of Pittenger et al (Science, Vol. 284, pages 143-147, 1999, cited in a prior action; see the entire reference). This rejection has been rewritten to address the amendments to the claims in the reply filed 7/19/2011.

The combined teachings of Caplan et al, Burkitt et al, and Matsui et al are described above and applied as before.

Caplan et al, Burkitt et al, and Matsui et al do not specifically teach that the mesenchymal stem cells are CD34-.

Pittenger et al teach the isolation of human mesenchymal cells from bone marrow taken from the iliac crest (e.g., page 143, right column). Pittenger et al teach that the mesenchymal stem cells are CD34- (e.g., paragraph bridging pages 143-144). The mesenchymal stem cells isolated by Pittenger et al are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to specifically use a bone marrow aspirate from human iliac crest, because Caplan et al and Pittenger et al teach the use of bone marrow from iliac crest to isolate mesenchymal stem cells that are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute iliac crest bone marrow for any other type of bone marrow to achieve the predictable result of recovering CD34- mesenchymal stem cells that are also capable of differentiating to adipose, cartilage or bone tissue.

Claims 1, 4, 6, 9, 11, 34-38 and 42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Guirguis (US Patent No. 5,077,012, cited in a prior action; see the entire reference) and Matsui et al (US Patent No. 4,871,674, cited in a prior action; see the entire

reference). This rejection has been rewritten to address the amendments to the claims in the reply filed 7/19/2011.

Caplan et al teach the isolation of human mesenchymal stem cells from aspirated marrow, comprising the steps of (i) applying the cells to a Percoll gradient and collecting the low density platelet fraction containing marrow-derived mesenchymal stem cells, platelet cells, and red blood cells; (ii) placing the cells in complete medium; (iii) allowing the cells to adhere to the surface of Petri dishes for one to seven days; and (iv) removing non-adherent cells after three days by replacing the original complete medium with fresh complete medium, thereby providing a homogenous population of human mesenchymal stem cells free of markers associated with hematopoietic cells (e.g., column 1, line 56 to column 3, line 19; column 11, line 63 to column 12, line 25). Caplan et al teach that complete medium and Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1 g/L of glucose stimulates mesenchymal stem cell growth without differentiation and allows for the selective attachment of only mesenchymal stem cells to the plastic surfaces of the Petri dishes (e.g., column 8, line 45 to column 9, line 55; column 45, line 45 to column 46, line 34). Caplan et al teach that mesenchymal stem cells can be grown until the culture dishes become confluent (e.g., paragraph bridging columns 19-20). Caplan et al teach that when the culture dishes become confluent, the cells are detached with 0.25% trypsin with 0.1 mM EDTA for 10-15 minutes at 37° C, the action of trypsin is stopped with fetal bovine serum, the cells are counted, split 1:3 and replated in 7 ml of complete medium (e.g., paragraph bridging columns 19-20; paragraph bridging columns 40-41). Caplan et al teach plating the recovered cells into 35 mm plates at 50,000 cells, which is a density of about 5 x $10^3/cm^2$ (e.g., column 41). Caplan et al teach that the mesenchymal stem cells can differentiate

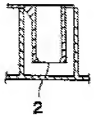
into bone, cartilage or adipose tissue (e.g., column 1, lines 40-52; column 47, lines 9-48). Moreover, Caplan et al teach that a porous filter can be used to remove red blood cells from the mesenchymal stem cells to provide an enriched population of mesenchymal stem cells (e.g., column 45, line 45 to column 46, line 34).

Caplan et al do not teach the method of isolating human mesenchymal stem cells where the mixed population of cells in medium is seeded into a culture device comprising an upper plate with pores and a lower plate base, where small cells pass through the pores in the upper plate and adhere to the lower plate.

Guirguis teaches the removal of red blood cells from a body fluid using a membrane with a smooth flat surface which is ideal for the collection of atypical cells from all types of body fluids (e.g., column 3, lines 37-45; column 4). Guirguis et al teach that the membrane has a preferred pore size of 2 microns or less (e.g., column 4, lines 14-19). Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity (e.g., column 4, lines 43-64).

Matsui et al teach culturing cells in a cell culture device comprising a cell culture insert comprising a membrane filter (2) on the bottom of the culture cell, which is composed of polycarbonate (e.g., column 2, lines 41-55; column 3, lines 5-18). The culture device is shown in Figure 8, which is reproduced below:

Fig. 8



It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the method of isolating mesenchymal stem cells of Caplan et al to include the introduction of the mixed composition of cells comprising mesenchymal stem cells and medium into the culture dish taught by Matsui et al because Caplan et al teach it is within the ordinary skill in the art to culture mesenchymal stem cells on plastic and teach the use a filter to remove fat cells and red blood cells from cells of bone marrow. Furthermore, Guirguis teaches the use of a polycarbonate membrane for the removal of red blood cells from a body fluid, and Matsui et al teach culturing cells in a device comprising a polycarbonate filter.

One would have been motivated to make such a modification in order to receive the expected benefit of providing an enriched population of mesenchymal stem cells without having to perform the extra steps of using a separate filter as taught by Caplan et al, since red blood cell removal and mesenchymal stem cell culture could be performed simultaneously using the culture dish of Matsui et al. Further, one would have been motivated to use the polycarbonate (plastic) filter in place of the Leukosorb filter taught by Caplan et al, because Caplan et al teach that mesenchymal stem cells become selectively attached to plastic in DMEM containing 10% FBS and 1 g/L of glucose or complete medium, and Guirguis teaches that the advantage of using a polycarbonate membrane is the minimum clogging by red blood cells and protein, well preserved

cellular morphology with a high recovery rate, and excellent surface capture due to the pore structure and porosity of the polycarbonate. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Caplan et al (US Patent No. 5,811,094, cited in a prior action; see the entire reference) in view of Guirguis (US Patent No. 5,077,012, cited in a prior action; see the entire reference) and Matsui et al (US Patent No. 4,871,674, cited in a prior action; see the entire reference) as applied to claims 1, 4, 6, 9, 11, 34-38 and 42 above, and further in view of Pittenger et al (Science, Vol. 284, pages 143-147, 1999, cited in a prior action; see the entire reference). This rejection has been rewritten to address the amendments to the claims in the reply filed 7/19/2011.

The combined teachings of Caplan et al, Guirguis et al, and Matsui et al are described above and applied as before.

Caplan et al, Guirguis et al, and Matsui et al do not specifically teach that the mesenchymal stem cells are CD34-.

Pittenger et al teach the isolation of human mesenchymal cells from bone marrow taken from the iliac crest (e.g., page 143, right column). Pittenger et al teach that the mesenchymal stem cells are CD34- (e.g., paragraph bridging pages 143-144). The mesenchymal stem cells isolated by Pittenger et al are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to specifically use a bone marrow aspirate from human iliac crest, because Caplan et al and Pittenger et al teach the use of bone marrow from iliac crest to isolate mesenchymal stem cells that are capable of differentiating to adipose, cartilage or bone tissue (e.g., Figure 2). It would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute iliac crest bone marrow for any other type of bone marrow to achieve the predictable result of recovering CD34- mesenchymal stem cells that are also capable of differentiating to adipose, cartilage or bone tissue.

Response to Arguments - 35 USC § 103

With regard to the above rejections under 35 USC 103(a), Applicant's arguments filed 7/19/2011 have been fully considered but they are not persuasive.

The response asserts that the limitation of "recovering with trypsin-EDTA" distinguishes the claimed invention from the method using a Leukosorb filter as taught by Caplan. The response notes that the elution of cells from the Leukosorb filter does not require trypsin-EDTA, and Caplan use trypsin-EDTA to detach cells from culture dishes. Thus, the response asserts that the present claims are different from the combination of Caplan's, Prockop's, and Matsui's teachings.

This argument is not found persuasive. The rejection of record is based upon the combined teachings of the reference where a Leukosorb filter is not used to remove red blood cells; rather, a tissue culture dish containing pores is used to remove the red blood cells. As noted by Applicant, Caplan does teach the use of trypsin-EDTA to detach the mesenchymal stem

cells from the culture dish. Thus, one of skill in the art would have known to use trypsin-EDTA to recover the mesenchymal stem cells from the culture dish containing pores. One would have been motivated to use a known method to collect the cells in order to successfully collect the cells for replating as taught by Caplan.

The response asserts that the present application discloses unexpected results, which are recited in claims 43-45. The response asserts that the unexpected results in the proliferation of the mesenchymal stem cells to confluence without differentiation even after 12 passages. Further, the reply cites the post-filing art (Kato) to show that the proliferation of mesenchymal stem cells becomes extremely slow around the 15^{th} generation.

This argument is not found persuasive. Claims 43-45 are drawn to a nonelected invention and are withdrawn from consideration. The claims currently under consideration do not require any particular number of cell passages and do not require 12 passages, where the cells grow to confluence without differentiation. Furthermore, the passage of Kato cited by Applicant seems to indicate that the cells are capable of proliferating to confluence at passage 12 without difficulty.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is (571)272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ardin Marschel can be reached on 571-272-0718. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Jennifer Dunston/
Primary Examiner
Art Unit 1636